Reactions of Fe(II)-ATP and Fe(II)-citrate complexes with *t*-butyl hydroperoxide and cumyl hydroperoxide

J.D. Rush and W.H. Koppenol

Biodynamics Institute and Departments of Chemistry and Biochemistry, Louisiana State University, Baton Rouge, LA 70803-1804, USA

Received 21 September 1990

Rate constants for the reactions of cumyl hydroperoxide and *t*-butyl hydroperoxide with ferrous complexes of ATP and citrate were measured at pH 7.4. These ligands are potential chelators of iron(II) in the low-molecular weight iron pool that may catalyze oxidative degradation of biomolecules. The second-order rate constants for the reduction of cumyl hydroperoxide and *t*-butyl hydroperoxide by ferrous ATP are 3.1×10^3 and 1.3×10^3 $M^{-1} \cdot s^{-1}$, respectively, at 25°C and 0.11 M ionic strength. Rates of reduction by ferrous citrate are similar. Activation enthalpies for these reactions average 10 kcal/mol.

Fenton reaction; Adenosine triphosphate; Citrate, Cumyl hydroperoxide; *t*-Butyl hydroperoxide; Ferrous nucleotide; Alkoxyl radical

1. INTRODUCTION

Recently, we reported rate constants for the reactions of iron (II)-nucleotide complexes with hydrogen peroxide [1]. These rate data indicated that the reaction proceeded faster when the iron(II) complex had more coordination sites occupied by water, in line with the presumed inner-sphere character of this electron transfer reaction. This trend is similar to that observed in a series of aminopolycarboxylate ligands [2,3]. If indeed steric factors contribute to the rate of reaction, then one would expect alkyl hydroperoxides to react more slowly than hydrogen peroxide.

It has been shown that iron bound to ATP and AMP [4], and possibly citrate [5], constitutes the major part of the small concentration of low-molecular weight iron complexes in vivo. This pool of soluble iron is potentially harmful in that it can catalyze reactions that result in reactive oxyradicals [1,6]. Three such reactions are relevant in this context: (i) the autoxidation of ferrous complexes yields superoxide which, although not very reactive in water, is a precursor of more reactive radical species; (ii) the one-electron reduction of hydrogen peroxide results in hydroxyl radicals directly, or via the ferryl intermediate [1,3], and (iii) the one-electron reduction of alkyl hydroperoxides leads to alkoxyl radicals. The hydroxyl radical is a powerful oxidant, $E^{\circ\prime}(^{\bullet}OH/H_2O) = 2.31$ V at pH 7 [7], that reacts indiscriminately with biomolecules at diffusion-controlled rates [8]. Alkoxyl radicals are less oxidizing, $E^{\circ\prime}(^{\bullet}RO/ROH) = 1.6$ V [9]. Rate constants for the reactions mentioned above are essential to determine which reaction will dominate under physiological conditions, and to calculate rates of radical production. The topic of this communiation is the third reaction. The organic hydroperoxides selected for this study showed similar reactivities towards ferrous complexes. It seems reasonable that these results also apply to alkyl hydroperoxides that are of a more physiological nature.

Correspondence address: W.H. Koppenol, Biodynamics Institute, Louisiana State University, Baton Rouge, LA 70803-1800, USA

2. MATERIALS AND METHODS

Kinetic data were obtained with a stopped-flow spectrophotometer (Kinetics Instruments/On-Line Instruments Systems) maintained at a constant temperature with a VWR 1160 circulation bath. Buffered solutions with the hydroperoxide and free ligand - ATP or citrate - were mixed with a ferrous ammonium sulphate solution, and the formation of iron(III) was monitored at 300 nm. 10-fold stoichiometric excesses of cumyl or *t*-butyl hydroperoxide over iron(II) were used and all solutions contained 0.1 M NaCl and 5 mM phosphate (pH = 7.4). Identical rate constants were obtained when iron(II) was the excess reagent. ATP and citrate concentrations were at least in 5-fold excess and of sufficient concentration (>1 mM) to completely complex iron(II). First-order rate constants were determined at 25°C. Activation energies were obtained by determining the effect of temperature on the rate of reaction at one concentration of alkyl hydroperoxide.

Cumyl hydroperoxide, $C_6H_5(CH_3)_2COOH$, and *t*-butyl hydroperoxide, $(CH_3)_3COOH$, were from Aldrich. Aquous stock solutions were prepared and quantified by the oxidation of iodide to iodine (I_3^-). The disodium salt of adenosine 5'-triphosphate was from Sigma. All other common chemicals were of analyzed reagent quality and the water was purified by reverse osmosis, deionization and filtration (Marcor).

3. RESULTS

The observed rates of iron(III) formation for reactions 1-4 as a function of alkyl hydroperoxide concentration, are shown in Fig. 1. The reactions are first-order in both reactants and the observed rates are independent of the excess reagent, which indicates that

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00145793/90/$3.50

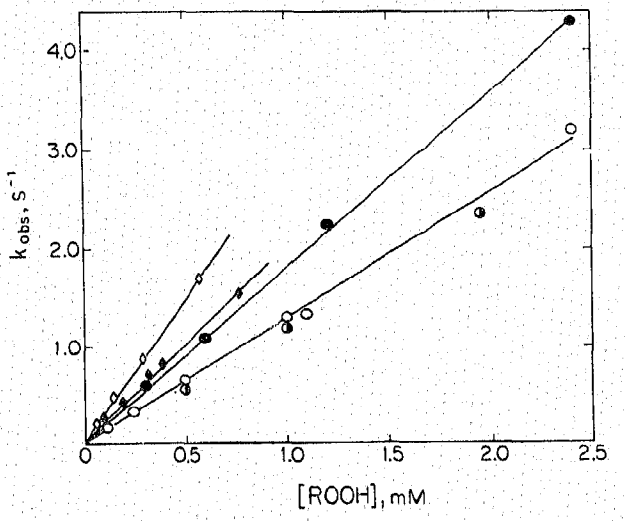

Fig. 1. The dependence of the observed rate constants for iron(III) formation upon alkyl hydroperoxide concentrations at pH 7.4, t = 25°C: (◇) $Fe^{II}ATP$ + cumyl-OOH; (○) $Fe^{II}ATP$ + t-butyl-OOH; (◆) Fe^{II}citrate + cumyl-OOH; (●) Fe^{II}citrate + t-butyl-OOH. Other data points indicated (⊖) are for the reaction of $Fe^{II}ATP$ + t-butyl-OOH run with an excess of the iron(II) complex. At concentrations of cumyl hydroperoxide in excess of approx. 1 mM, the apparent rate constant decreases, probably due to micelle formation.

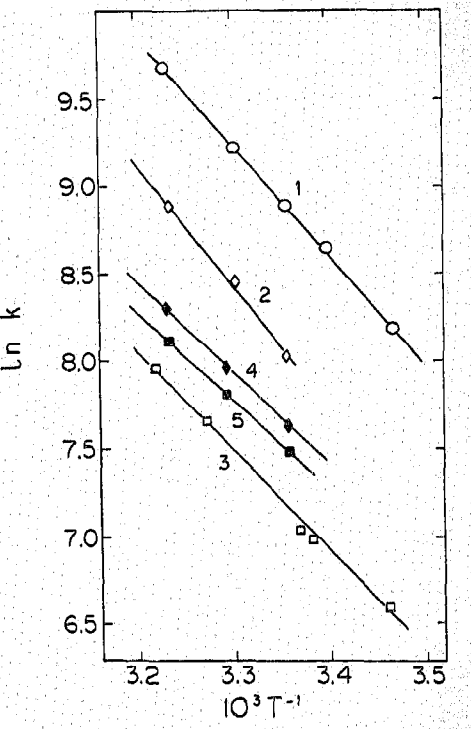

Figure 2. The temperature dependences of second-order rate constants at pH 7.4 for the reactions LFe^{II} + ROOH. 1, L = ATP, R = H; 2, L = ATP, R = cumyl; 3, L = ATP, R = t-butyl; 4, L = citrate, R = cumyl; 5, L = citrate, R = t-butyl.

the number of iron(II) complexes oxidized per hydroperoxide consumed in invariant. In the case of cumyl hydroperoxide the stoichiometry is 1 because the cumoxyl radical decomposes to acetophenone and a methyl radical, as shown in Eqn 1. t-Butyl hydroperoxide was found to produce approx. two Fe(III), which indicates that the t-butoxyl radical is reactive towards excess iron(II) complex see Eqns 2 and 4).

$$Fe^{II}ATP + C_6H_5(CH_3)_2COOH + H^+ \xrightarrow{k_1} Fe^{III}ATP + C_6H_5COCH_3 + {}^{\bullet}CH_3 \quad (1)$$

$$2\ Fe^{II}ATP + (CH_3)_3COOH + 2\ H^+ \xrightarrow{k_2} 2\ Fe^{III}ATP + (CH_3)_3COH \quad (2)$$

$$Fe^{II}citrate + C_6H_5(CH_3)_2COOH + H^+ \xrightarrow{k_3} Fe^{III}citrate + C_6H_5COCH_3 + {}^{\bullet}CH_3 \quad (3)$$

$$2\ Fe^{II}citrate + (CH_3)_3COOH + 2\ H^+ \xrightarrow{k_4} 2\ Fe^{III}citrate + (CH_3)_3COOH \quad (4)$$

The rate-limiting step in both cases is the one-electron reduction of the alkyl hydroperoxide by the iron(II) complex in Eqn 5, followed by the fast oxidation of iron(II) by the resultant alkoxyl radical (if stable) in Eqn 6, as observed in Eqns 2 and 4, and in the reaction with cumylhydroperoxide with ferrous pyrophosphate at neutral pH [10].

$$Fe^{II}ATP + ROOH \rightarrow Fe^{III}(OH^-)ATP + RO^{\bullet} \quad (5)$$

$$Fe^{II}ATP + H_2O + RO^{\bullet} \rightarrow Fe^{III}(OH^-)ATP + ROH \quad (6)$$

The alkoxyl radical does not react with dioxygen and the reaction is therefore not affected by the presence or absence of dioxygen. This radical does not attack organic components in competition with Eqn 6 as readily as does the hydroxyl radical, which can initiate chain reactions unless the reactions are monitored under anaerobic conditions.

Temperature dependences (ln k vs T^{-1} for reactions 1–4, as well as for the reaction of $Fe^{II}ATP$ with hydrogen peroxide, are shown in Fig. 2. Activation enthalpies of the alkyl hydroperoxide reduction reactions average 10 kcal/mol. These data and those from Fig. 1 are summarized in Table I, along with published data for the reduction of hydrogen peroxide by these complexes [1].

4. DISCUSSION

Rate constants for reductions of cumyl hydroperoxide with Fe(II) at low pH, 16 $M^{-1}\cdot s^{-1}$ [11,12], and with the Fe(II)EDTA at neutral pH, 1.1×10^3 $M^{-1}\cdot s^{-1}$ [10], are smaller than those reported for the corresponding reaction with hydrogen peroxide, 42 $M^{-1}\cdot s^{-1}$ [13] and 7.0×10^3 $M^{-1}\cdot s^{-1}$ [2,14] by a factor of 3–6. A larger factor is observed if one compares the reductions of cumyl hydroperoxide and hydrogen peroxide by ferrous pyrophosphate at neutral pH, 0.83×10^3 $M^{-1}\cdot s^{-1}$ [15], respectively. Ferrous ions liganded by bipyridine and water showed aproximately equal reactivity towards

Table 1

Rate constants and activation energies for reactions of ferrous ATP and ferrous citrate complexes with H_2O_2, cumyl- and *t*-butyl hydroperoxide

Reaction	k^a($M^{-1}s^{-1}$)		$\Delta H^{\ddagger}$(kcal/mol)
	25°C	37°C	
Fe^{II}ATP + ROOH			
R = H	6.7×10^{3}	1.6×10^{4}	11.5
R = *t*-butyl	1.3×10^{3}	2.7×10^{3}	10.6
R = cumyl	3.1×10^{3}	6.5×10^{3}	12.4
Fe^{II}citrate + ROOH			
R = H	4.9×10^{3}	n.d.	n.d.
R = *t*-butyl	1.8×10^{3}	3.4×10^{3}	8.9
R = cumyl	2.2×10^{3}	4.2×10^{3}	9.4

[a] All rate constants were obtained at pH 7.4 in a 0.1 M NaCl medium

hydrogen peroxide, cumyl hydroperoxide and *t*-butyl hydroperoxide [16]. We find that ferrous ATP and ferrous citrate react slower with cumyl hydroperoxide and *t*-hydroperoxide than with hydrogen peroxide by a factor of 2–5. This is in part accounted for by a statistical factor of 2, since H_2O_2 has two reactive $^{\cdot}OH$ sites compared to one in the alkyl hydroperoxides. The modest decrease in rate which results form the alkyl substitution indicates quite clearly that only one of the hydroperoxide oxygens is involved in the transition state complex that precedes electron transfer. We have previously observed in reactions of ferrous aminopolycarboxylate complexes with hydrogen peroxide that the rate increases when at least one coordination site is occupied by water, compared to a hexa- or hepta-coordinate ligand such as EDTA or DTPA. Additional liganded water molecules do not significantly increase the rate [2,3]. Hence, the transition state in hydroperoxide reduction is presumed to be a ferrous-hydroperoxide intermediate involving a single iron-to-oxygen bridge [17,18].

$$\text{L-Fe}^{II}(H_2O) + HOOR \leftrightarrow \text{L-Fe}^{II}\text{-}\overset{H}{\underset{|}{O}}\text{OR} \rightarrow \text{L-Fe}^{III}\text{-OH}^{-} + {}^{\cdot}OR \qquad (7)$$

The similarity in activation enthalpies also suggests that possible differences in hydroperoxide bond strengths are not very important, although a relation between bond strengths in a series of stubstituted cumyl hydroperoxides and activation enthalpies was established [17]. In our studies activation energies and rate constants appear to depend more on the iron complex than the species of hydroperoxide. The one-electron reduction of an alkylhydroperoxide is approximately 38 kcal/mol more favourable than the corresponding reduction of hydrogen peroxide [9]. It is clear that the overall energetics do not influence the rate of reaction. ESR-flow studies show that iron(II) reduces 13-L-hydroperoxylinoleic acid with a rate constant of 33 $M^{-1}\cdot s^{-1}$ at 25°C, which is comparable to 16 $M^{-1}\cdot s^{-1}$ [12,19] at 25°C and pH 1 for the one-electron reduction of cumyl hydroperoxide. It seems reasonable, therefore, to assume that rate constants reported here for pH 7 also apply to hydroperoxides with longer alkyl chains, which are more relevant to oxidative processes in vivo. The study of more physiological alkylhydroperoxides is difficult due to their hydrophobic nature, as illustrated by a report on the decomposition of phosphatidylcholine hydroperoxides in unilamellar vesicles by ferrous ions at pH 6. First-order kinetics in both reactants were observed and a rate constant of $(1.5\pm0.5)\times10^{3}$ $M^{-1}\cdot s^{-1}$ was determined [20]. However, this result obtained in an inhomogeneous system cannot be extrapolated to other conditions. This study suggests that the overall reactivity depends on the access of the hydroperoxide moiety to the aqueous phase, and that the intrinsic reactivity of the hydroperoxide group is relatively independent of the organic moiety.

Acknowledgement: This work was supported by a grant from the Council for Tobacco Research-USA, Inc.

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